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MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: NATURAL COURSE AND COMORBIDITIES

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Monoclonal Gammopathy of Undetermined Significance: Natural Course and Comorbidities
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Reinhold and Åsa.

ABSTRACT

Monoclonal gammopathy of undetermined significance (MGUS) is a plasma cell disorder characterized by an overproduction of monoclonal immunoglobulins. MGUS is asymptomatic but clinically relevant since annually 0.5-1.5% of individuals with MGUS will develop multiple myeloma (MM) or another malignant lymphoproliferative disease. Individuals with MGUS are followed for signs of progression, however, so far this management strategy has never been evaluated. Results from previous studies have shown that individuals with MGUS have inferior survival and increased risk of thrombosis compared to individuals without MGUS, yet all studies to date have been performed on clinically established cohorts of MGUS patients, introducing a high risk of selection bias. Recently, a new entity called light-chain MGUS (LC-MGUS) has been identified. Very little is known about the epidemiology and clinical course of LC-MGUS.

In order to establish a clinically informative, correct, and easily applicable definition of LC-MGUS, and describe the prevalence of MGUS and LC-MGUS in the population, we performed a large population-based screening study. We screened more than 11,000 individuals from the Icelandic AGES-Reykjavik Study cohort and the American PLCO Study cohort. The prevalence of MGUS was 4.8-5.2%. Based on findings from the two cohorts and on statistical analysis of normal distributions, we propose a revised definition of LC-MGUS; (1) an abnormal free light-chain ratio (<0.26 or >1.65), (2) an elevated involved light chain concentration (40 mg/L or higher), (3) no M-protein on serum protein electrophoresis or immunofixation, and (4) no evidence of end-organ damage that can be attributed to a lymphoproliferative disorder. The prevalence of LC-MGUS in our study using this definition was 0.9-1.0%. The prevalence of LC-MGUS increased with age ($p<0.001$), was higher in men ($p<0.001$), and more common among blacks (2.9%) than whites (0.7%) or Asian/Pacific Islanders (0.2%). The revised definition of LC-MGUS captures the condition in fewer but clinically relevant individuals.

We conducted three population-based studies with the purpose of studying the natural course and survival of individuals with MGUS and LC-MGUS. We used the Icelandic AGES-Reykjavik Study cohort of 5,764 individuals, including 300 individuals with MGUS and 52 individuals with LC-MGUS, as well as a Swedish cohort of 18,768 MGUS patients. Through the Swedish Cancer Register we identified all patients with MM diagnosed from 1976 to 2013, as well as randomly sampled population-based controls. Individuals with MGUS had a 1.2-fold (95% confidence interval (CI) 1.04-1.4) and individuals with LC-MGUS had a 1.6-fold (1.2-2.3) increased risk of death compared to individuals without MGUS, during a median follow-up time of almost ten years. The risk remained increased after progression to lymphoproliferative disease was taken into account. We found a personal history of autoimmune disease to increase the risk of death significantly in both individuals with MM (hazard ratio (HR) = 1.2, 1.2-1.3) and individuals with MGUS (HR = 1.4, 1.3-1.4). These findings could be due to an underlying genetic susceptibility for both plasma cell disorders and other conditions, such as autoimmune disease, or to the overproduction of light chains causing organ damage. We found that MM patients with prior knowledge of MGUS had a better overall survival (median survival 2.8 years) than MM patients without prior knowledge of MGUS (median survival 2.1 years). Among MM patients with a prior knowledge of MGUS, a low M-protein concentration at MGUS diagnosis was predictive of worse survival in MM (HR = 1.9, 1.1-3.0), possibly due to patients with low M-protein concentration being followed less frequently. Our findings support the recommendations of regular clinical follow-up of individuals with MGUS, regardless of M-protein concentration.

In further analysis of the AGES-Reykjavik Study cohort, we assessed the causes of death and risk of thrombosis among individuals with MGUS and LC-MGUS and found an increased risk of death from cancer (HR = 1.8, 1.6-2.3) and from heart disease (HR = 1.4, 1.1-1.8), adjusted for age and sex. We found that a history of thrombosis was more common in individuals with LC-MGUS (25%) than individuals with MGUS (10%) or without MGUS (12%), and that individuals with LC-MGUS had an increased risk of a history of arterial thrombosis especially (crude odds ratio (OR) = 2.5, 95% CI 1.3-4.9), compared to individuals without MGUS. During a median follow-up time of almost nine years, we detected an almost two-fold risk of arterial thrombosis in individuals with LC-MGUS compared to individuals without MGUS (crude HR = 1.9, 1.1-3.2). No increased risk of venous thrombosis was detected in individuals with MGUS or LC-MGUS. Our results suggest that previously detected increased risks of thrombosis in MGUS have been due to confounding factors. Our findings on LC-MGUS point towards an elevated risk of arterial, but not venous, thrombosis.

In future investigations, we suggest attention is focused on characterizing the clinical, genetic, and biochemical profiles of LC-MGUS, with the purpose of understanding the connection to cancer, to heart disease, and to thrombosis.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Kristinsson SY, **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Hoffman JN, Purdue MP, Mailankody S, Steingrimsdottir H, Murata K, Björkholm M, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Gudnason V, Landgren O. *Light-chain monoclonal gammopathy of undetermined significance: a new definition.* Manuscript 2016.

II. **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Mailankody S, Björkholm M, Gudnason V, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Landgren O, Kristinsson SY. *The increased risk of dying in individuals with monoclonal gammopathy of undetermined significance is caused by malignant progression and heart disease.* Manuscript 2016.

III. **Lindqvist EK**, Landgren O, Lund SH, Turesson I, Hultcrantz M, Goldin L, Björkholm M, Kristinsson SY. *History of autoimmune disease is associated with impaired survival in multiple myeloma and monoclonal gammopathy of undetermined significance: a population-based study.* Ann Hematol. 2016 Nov 2. [Epub ahead of print]

IV. Sigurdardottir EE, Turesson I, Lund SH, **Lindqvist EK**, Mailankody S, Korde N, Björkholm M, Landgren O, Kristinsson SY. *The role of diagnosis and clinical follow-up of monoclonal gammopathy of undetermined significance (MGUS) on survival in multiple myeloma.* JAMA Oncol. 2015;1(2):168-174.

V. **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Mailankody S, Björkholm M, Gudnason V, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Landgren O, Kristinsson SY. *Monoclonal gammopathy of undetermined significance and risk of arterial and venous thrombosis: results from a population-based study.* Manuscript 2016.

CONTENTS

LIST OF ABBREVIATIONS

AGES	Age, Gene/Environment Susceptibility study
CI	Confidence interval
FLC	Free light chain
HR	Hazard ratio
IFE	Immunofixation electrophoresis
Ig	Immunoglobulin
LC-MGUS	Light-chain monoclonal gammopathy of undetermined significance
MGUS	Monoclonal gammopathy of undetermined significance
MM	Multiple myeloma
M-protein	Monoclonal protein
OR	Odds ratio
PLCO	Prostate, Lung, Colon and Ovarian Cancer study
SPEP	Serum protein electrophoresis

1 INTRODUCTION

1.1 LYMPHOPROLIFERATIVE DISORDERS

All cells of the blood are derived from common hematopoietic progenitor cells that reside mainly in the bone marrow and give rise to lymphoid, myeloid, erythroid, monocyte, and megakaryocyte lineages.[1-3] The cell designated for the lymphoid pathway will eventually develop into a B lymphocyte, a T lymphocyte, an NK-cell or a dendritic cell. Lymphoproliferative disorders such as chronic lymphocytic leukemia and lymphoma are cancers of the blood and the lymph nodes, derived from cells in different stages of the lymphoid pathway, and a majority of these malignancies stem from B lymphocytes.[4,5]

B lymphocytes from the bone marrow differentiate into plasma cells that produce immunoglobulins, commonly known as antibodies, and are the basis of the humoral immune system.[1,2,6,7] Immunoglobulins consist of two heavy polypeptide chains of either gamma, alpha, mu, delta, or epsilon type, and two light polypeptide chains of either kappa or lambda type (Figure 1).[7,8]

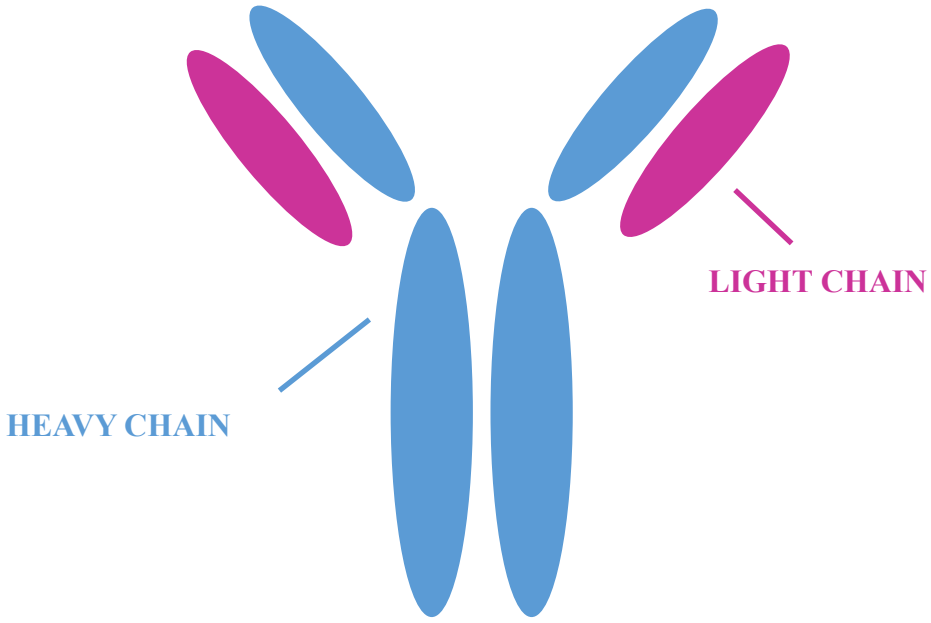


Figure 1. Basic structure of an immunoglobulin (antibody) with heavy chains (blue) and light chains (purple).

Under normal circumstances, a large diversity of plasma cells are present in the blood, secreting a variety of immunoglobulins – polyclonal secretion.[9] In plasma cell disorders, a clonal proliferation of plasma cells leads to overproduction of a single clone of immunoglobulins – so called monoclonal immunoglobulins.[4,10] The monoclonal overload in the blood can be detected as a monoclonal band, known as an M-protein, on serum protein electrophoresis (SPEP).[4] The clinical manifestations of different plasma cell disorders are due to the expansion of neoplastic cells in the bone marrow, in the blood, or in other lymphatic or non-lymphatic organs, and to the secretion of immunoglobulins. In Bence Jones proteinuria, monoclonal light chains are secreted in the urine and can be detected by urine protein electrophoresis or urine immunofixation.[11,12]

1.2 MULTIPLE MYELOMA

1.2.1 Definition and epidemiology

Multiple myeloma (MM) is the most common lymphoproliferative disorder after non-Hodgkin lymphoma, and accounts for about 10% of all hematological malignancies.[13,14] Worldwide incidence rates vary from 0.7 to 3.3 per 100,000 person-years.[13,15,16] MM is characterized by a monoclonal proliferation of plasma cells in the bone marrow (Figure 2), M-protein in the blood or urine, and end-organ damage such as anemia.[4,5,10] Common clinical manifestations among patients include fatigue and bone pain, due to underlying anemia and osteolytic lesions.[17,18] MM is a disease of the elderly, with a median age at diagnosis of approximately 70 years.[19,20] It is more common in African-Americans than in Caucasians, and men are more frequently affected than women.[15,21,22]

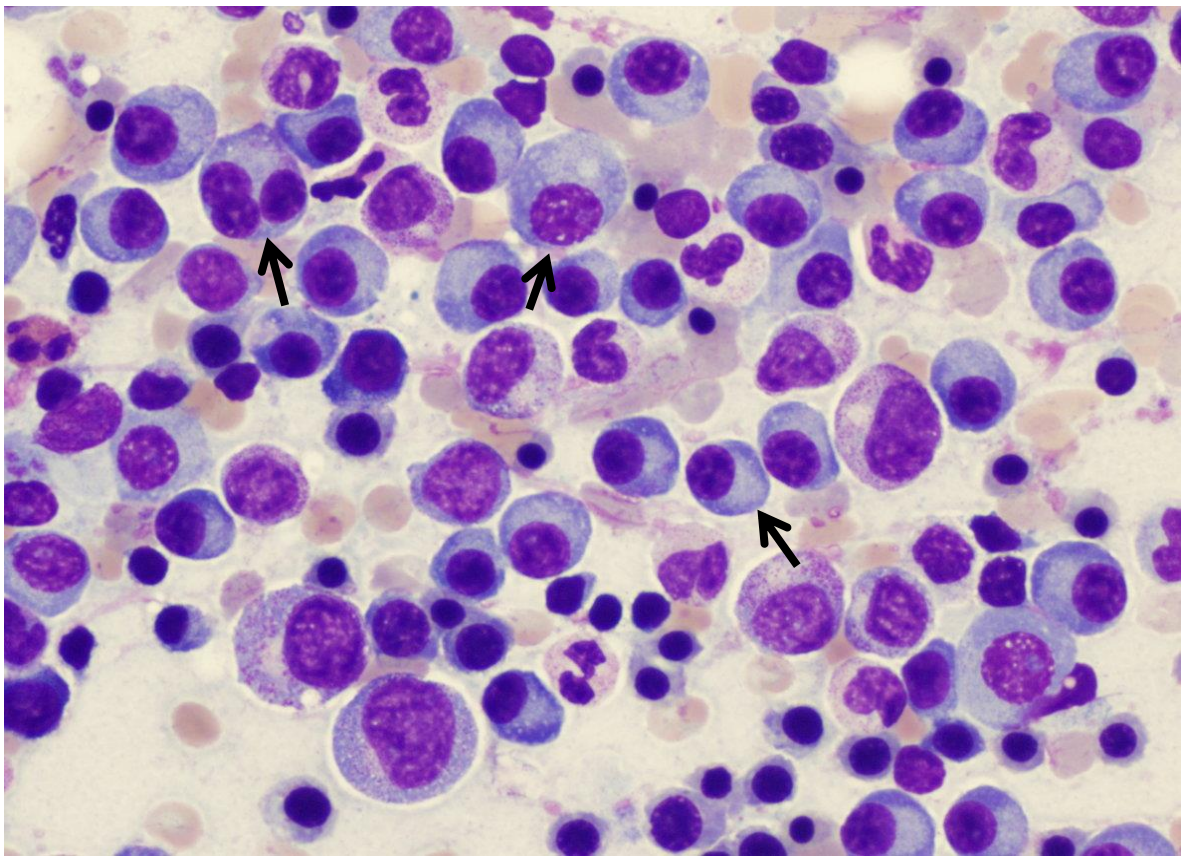


Figure 2. A picture of the bone marrow from a patient with multiple myeloma. The bone marrow is infiltrated with plasma cells/plasma blasts of different maturation (arrows).

The International Myeloma Working group diagnostic criteria for MM have recently been updated and now include at least 10% of clonal plasma cells in the bone marrow, presence of a myeloma defining event, or specific biomarkers (Table 1). An early stage of MM is smoldering MM, when the patient has no myeloma defining events, but a serum M-protein

of ≥30 g/L or urinary M-protein ≥500 mg per 24 hours, and/or clonal bone marrow plasma cells between 10 and 60%.[17]

Table 1. Diagnostic criteria for multiple myeloma[17]	
1) Clonal bone marrow plasma cells ≥10%	Or biopsy-proven bony or extramedullary plasmacytoma
and	
2) Myeloma defining events: evidence of end organ damage such as:	Hypercalcemia
	Renal insufficiency
and	Anemia
	Bone lesions
3) Biomarkers of malignancy:	Clonal bone marrow plasma cells ≥60%
	Involved:uninvolved serum free light chain ratio ≥100
	>1 focal lesions on MRI studies[a]

[a]MRI: magnetic resonance imaging

1.2.2 Etiology and pathogenesis

The etiology of MM is largely unknown. However, there is evidence for a role of genetic factors, such as studies showing familial aggregation of MM,[23-28] racial disparities in incidence,[21,22] and germline single-nucleotide polymorphisms that have been associated with an increased risk of developing MM.[29] Both autoimmune disease and infectious disease have been linked to an increased risk of developing MM, suggesting chronic or powerful immune system stimulation could play a role.[24,30-32] Furthermore, dietary factors have been investigated, and the consumption of fish and seafood has been shown to decrease the risk of MM, suggesting that environmental factors also play a part in the etiology.[33]

The development of MM is an intricate process involving genetic changes in the plasma cell as well as in the bone marrow microenvironment.[34] Early hits in myelomagenesis include either chromosome 14 (*IGH* locus) translocations or hyperdiploidy, which are observed at a very early stage.[35] Cytogenetic aberrations are evaluated in routine clinical practice for risk stratification, see below.[36] Furthermore, recent sequencing studies have revealed a complex genetic landscape in MM including somatic mutations in *KRAS, NRS, FAM46C, BRAF, TP53, TRAF3, DIS3, CYLD*, and more.[37-39] Recently, a large genome-wide association study confirmed nine previously known risk loci and discovered eight new loci.[40] Epigenetic abnormalities, including deregulation of methylation pathways, have also been shown to contribute to the initiation or the progression of MM.[41]

MM is consistently preceded by a precursor condition called monoclonal gammopathy of undetermined significance (MGUS), see below.[42,43]

1.2.3 Treatment and prognosis

The cornerstone of MM treatment was for a long time melphalan-prednisone; a combination of an alkylating agent and a corticosteroid leading to a median overall survival of between two and four years.[44-46] In the last fifteen years, the treatment arsenal has been expanded with novel agents, such as proteasome inhibitors, immunomodulatory agents such as thalidomide, lenalidomide, and pomalidomide, interferons, monoclonal antibodies, histone deacetylase inhibitors, and kinesin spindle protein inhibitors, as well as with stem cell transplantation.[47-52] As a result, survival in MM has improved significantly, and median overall survival is now more than six years.[53-56] In 2015, three new agents for multiple myeloma were approved by the US Food and Drug Administration; ixazomib, daratumumab, and elotuzumab, and treatment strategies are moving away from chemotherapy and towards novel agents only.[57]

A number of prognostic factors have been identified, such as type and concentration of the M-protein, C-reactive protein, albumin, β2-microglobulin, serum free light chains (FLC), and cytogenetic abnormalities.[58-62] The Revised International Staging System is a prognostic model for newly diagnosed MM patients, and takes into account albumin, β2-microglobulin, lactate dehydrogenase, and chromosomal abnormalities as detected by interphase fluorescent in situ hybridization, such as deletion(17p), translocation(4;14), and translocation(14;16).[63] Furthermore, it is well established that a higher comorbidity score, as measured by Freiburg Comorbidity Index, the Charlson Comorbidity Index, or the Hematopoietic Cell Transplant Comorbidity Index, predicts an inferior survival in patients with MM and is an independent risk factor for death even when the Revised International Staging System is taken into account.[64-69]

1.2.4 Thrombosis

The association between cancer and venous thrombosis is well established, leading to a higher mortality.[70-74] It has been suggested that neoplastic cells, possibly because of tumor cell hypoxia, activate the coagulation system by stimulating the production of various procoagulants and angiogenic factors such as tissue factor. Tumor cells as well as chemotherapeutic agents can injure endothelial cells which also activates the coagulation system.[71,75,76] Consequently, primary thromboprophylaxis with low molecular weight heparin is recommended to some, but not all, cancer patients.[72,73]

After the introduction of novel treatment agents for MM, an increased risk of venous thrombosis was observed, primarily in patients treated with immunomodulatory drugs.[77-80] Consequently, thromboprophylaxis with aspirin, low molecular weight heparin, or warfarin is recommended for MM patients who receive treatment regiments based on immunomodulatory drugs together with steroids.[80] It was initially believed that the increased risk of venous thrombosis in MM was solely a complication of the treatment. However, findings of an increased risk of venous thrombosis in the precursor state MGUS, and in MM patients before the introduction of novel agents, suggest that the plasma cell disorder in itself is also associated with an increased risk of thrombosis.[81,82] Furthermore, MM is associated with an elevated risk of arterial thrombosis, although this risk appears to be smaller than the

risk of venous thrombosis and has only been examined in two previous studies.[82,83] The increased risk of thrombosis in MM patients persists up to ten years after MM diagnosis, and has been demonstrated to affect survival negatively.[82,83]

Risk factors for venous thrombosis in MM include advanced age, obesity, personal or family history of venous thrombosis, presence of central venous catheter, trauma, surgery, immobility, use of tamoxifen or hormone replacement, thrombophilia, and comorbidities such as cardiac disease, renal impairment, or autoimmune disease.[80] More active disease and higher levels of M-protein have been linked to a stronger procoagulant state.[84,85] A variety of factors seem to be involved in venous thrombosis in MM, among them increased blood viscosity and inflammatory cytokines.[86] It appears that, similar to patients with other malignancies, patients with MM display changes in their coagulation status such as longer prothrombin times, and higher levels of D-dimer, factor VIII, tissue factor, fibrinogen, and von Willenbrand factor.[85,87]

1.3 MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

1.3.1 Definition

Monoclonal gammpathy of undetermined significance (MGUS) is a condition where there is an M-protein on SPEP without evidence of MM, amyloidosis, Waldenström macroglobulinemia, or other lymphoproliferative disorder (Figure 3).[5,17]

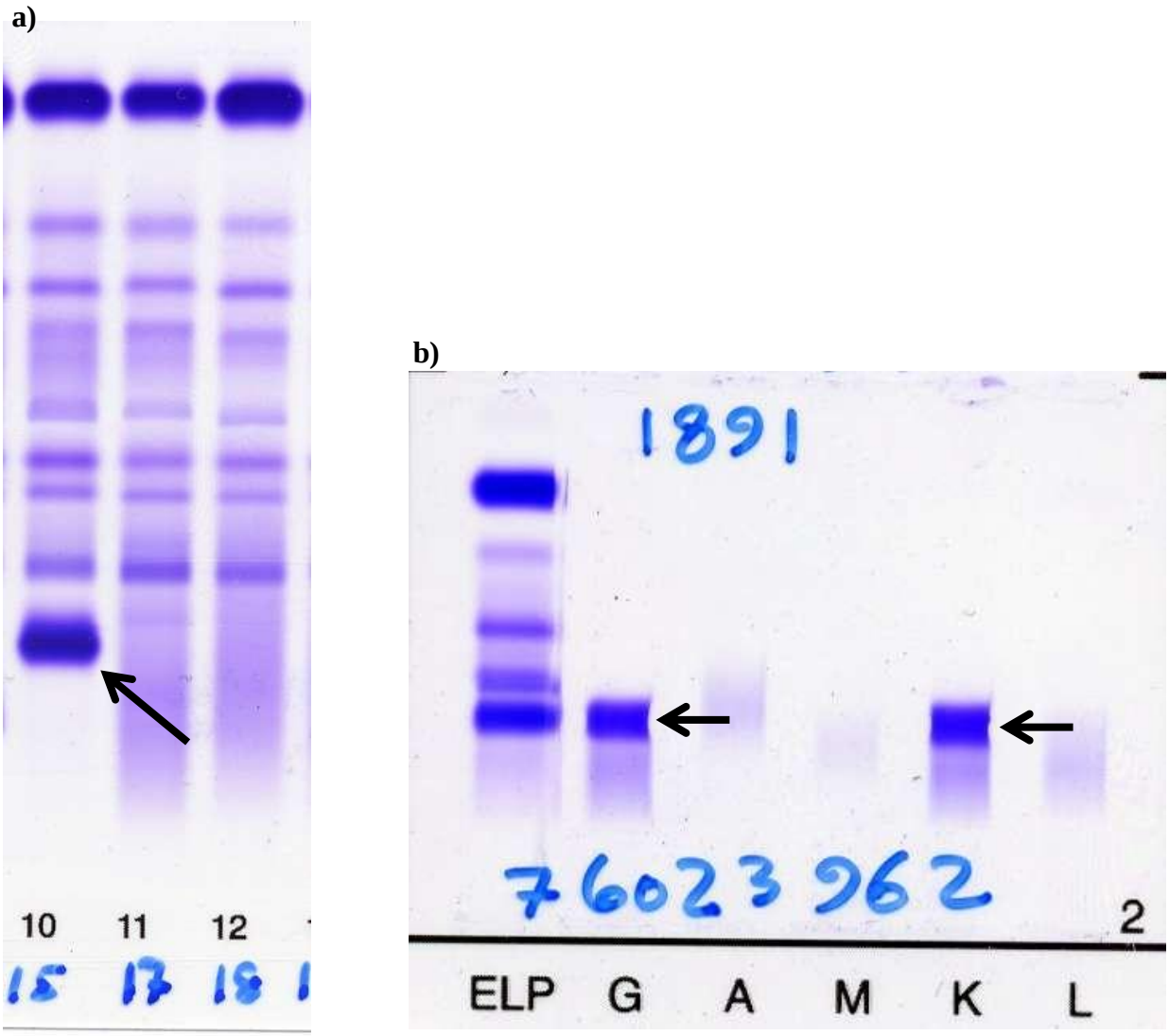


Figure 3. Serum electrophoresis panel (a) and immunofixation (b). The electrophoresis panel (a) contains one electrophoresis showing an M-protein (arrow), one with polyclonal hypergammaglobulinemia (middle), and one normal electrophoresis (right). The immunofixation (b) shows IgG kappa M-protein (arrows).

For the diagnosis of MGUS, the following is required: a serum M-protein concentration of less than 30 g/L, less than 10% of clonal plasma cells in the bone marrow, and absence of end-organ damage that can be attributed to a lymphoproliferative disorder (Table 2).[17]

Table 2. Diagnostic criteria for MGUS[17]	
Non-IgM MGUS:	
1) Serum M-protein <30 g/L	
2) Clonal bone marrow plasma cells <10%	
3) Absence of end-organ damage attributable to a lymphoproliferative disorder, such as:	Hypercalcemia
	Renal insufficiency
	Anemia
	Bone lesions
	Amyloidosis
IgM MGUS:	
1) Serum M-protein <30 g/L	
2) Bone marrow lymphoplasmacytic infiltration <10%	
3) Absence of end-organ damage attributable to a lymphoproliferative disorder, such as:	Anemia
	Constitutional symptoms
	Hyperviscosity
	Lymphadenopathy
	Hepatosplenomegaly

MGUS is in its nature asymptomatic, and so is the previously mentioned early stage of MM called smoldering MM. The difference between the two is in the concentration of M-protein in serum and the proportion of clonal plasma cells in the bone marrow, as detected through blood samples and bone marrow aspirations, respectively.[5,88] The M-protein secreted by the clonal plasma cells in MGUS determines the MGUS isotype, and can be of gamma (G), alpha (A), mu (M), delta (D), or epsilon (E) type, depending on the heavy chain of the immunoglobulin, in combination with kappa or lambda, depending on the light chain. In the diagnostic criteria, MGUS is divided into non-IgM MGUS and IgM-MGUS.[17] Recently, a new entity of MGUS called light-chain MGUS has been described (see below).

1.3.2 Epidemiology

Similar to MM, the prevalence of MGUS is highly associated with age. MGUS is present in 2.4-3.5% of white Caucasian populations over the age of 50 years.[22,89-93] According to findings from one study, the prevalence is as high as 5.3% in people older than 70 years.[89] MGUS appears to be more common in men than in women.[89,90] Due to its asymptomatic nature, cases of MGUS are usually found *en passant* during workup for some other condition, and the large majority of cases in the population are likely to go undetected. The only means to accurately estimate of the true prevalence of MGUS in the population is through screening. A few screening studies have been performed to date, on populations of different ethnicity, age and gender.[89,90,93 92] Kyle et al. screened 21,463 residents 50 years or older in Olmsted County, Minnesota, and found an MGUS prevalence of 3.2%.[89] Landgren et al. screened 917 men in Ghana and found a prevalence of 5.8%, and later screened 12,482 participants in the

NHANES and NHANES III studies and found a prevalence of 2.4%.[90,92] Finally, Eisele et al. determined the prevalence of MGUS to be 3.5% in a screened German population of 4,702 individuals.[93] Based on these findings, it seems reasonable to conclude that MGUS is a quite common, undetected condition among the elderly.

1.3.3 Etiology and pathogenesis

The etiology of MGUS is largely unknown. As in MM, there are ethnic disparities in the incidence patterns, where MGUS is more common in African-Americans than in Caucasians, and more common in Caucasians than in Asians.[22,90,92] Given the findings of increased risk of MGUS in first-degree relatives of patients with MM or MGUS, a role for genetic factors seems plausible.[25,94] Exposure to pesticides, such as Agent Orange, have also been linked to increased risk of developing MGUS, demonstrating an impact of environmental factors.[95,96] It has been shown that both a personal history of autoimmune disease, inflammatory condition, or infections, as well as a family history of autoimmune disease increase the risk of developing MGUS, suggesting a shared susceptibility for these conditions.[21,30] How autoimmune disease affects survival in MGUS is unknown.

1.3.4 Prognosis and survival

MM is consistently preceded by MGUS, however, not all individuals with MGUS develop MM or any other lymphoproliferative disorder.[42,43] The fact that MGUS can develop into a malignant disorder is what makes it clinically relevant, despite its asymptomatic state. At progression, non-IgM MGUS in the majority of cases evolves into MM, primary AL amyloidosis, or other lymphoproliferative disorders, and IgM MGUS progresses mainly to Waldenström macroglobulinemia.[97-100] The risk of progression to MM has been investigated in a few prospective studies, based on clinically established cohorts of individuals with MGUS. The Mayo cohort included 241 MGUS patients, followed up to 39 years. The overall risk of progression to any lymphoproliferative disorder was 1.5% per year, with a cumulative risk of 27%.[98] In another cohort of 1,384 MGUS patients from Minnesota, the annual risk of progression to any lymphoproliferative disorder was 1.0%, with a cumulative probability of progression of 30% at 25 years.[99] Results from a Swedish study following 728 MGUS patients showed an annual risk of progression to MM of 0.5%, with a 30-year cumulative risk of 10.6%.[97] In conclusion, the annual risk progression appears to be 0.5-1.0% per year.

Treatment is currently not recommended for MGUS. Expert opinions recommend indefinite follow-up of individuals with MGUS, with the hope of catching malignant progression early. The recommendations for follow-up are initially six months after diagnosis and then annually or every two to three years depending on risk stratification.[101,102] No investigators have shown results supporting these recommendations, such as better survival in MM after follow-up of MGUS.

According to the International Myeloma Working Group risk stratification of MGUS, low-risk MGUS is characterized by an M-protein <15 g/L, isotype IgG, and a normal FLC ratio (0.26-1.65 mg/L).[101] Additional risk stratification systems include the one presented by

Pérez-Persona et al., which defines low-risk MGUS as <95% bone marrow aberrant plasma cells, and no DNA aneuploidy, as assessed through flow cytometry, and the most recent scoring system developed by Turesson et al., in which low-risk MGUS is described by a normal FLC ratio, an M-protein <15 g/L, and presence of immunoparesis (reduction of noninvolved immunoglobulin isotype levels.)[97,103] Additionally, several other biological characteristics of the clone, along with detectable Bence Jones proteinuria and a high erythrocyte sedimentation rate (ESR), increase the risk of progression from MGUS to a lymphoproliferative disorder.[48,104]

As mentioned, the majority of individuals with MGUS will never develop a malignant disorder, and in that sense, MGUS has been considered a benign condition. However, results from studies on clinically established cohorts suggest that individuals with MGUS have a higher mortality.[98,100,105,106] A Dutch study found individuals with MGUS to have an inferior survival compared to population-based controls, even in the absence of progression to lymphoproliferative disorder, and also found serum albumin levels predictive of survival.[100] Gregersen et al. found that malignant transformation only explained around 20% of the excess mortality observed in their Danish cohort of MGUS patients, noting an increased mortality from several other causes of death including heart disease and lung disease, throughout the follow-up period.[105] In a large Swedish study, the 4,259 MGUS patients in the cohort had an increased risk of death not only from hematologic disorders, but also from bacterial infections, heart disease, liver disease, and renal disease, compared to matched controls.[55] However, these findings are all based on clinically established cohort of MGUS patients, and, as previously noted, cases of MGUS are usually detected during workup for some other disease. Consequently, clinically based MGUS cohorts are likely to have more comorbidities than the general population. The findings of decreased survival in these individuals are not necessarily due to MGUS in itself, but are just as likely to be explained by the underlying comorbidities that lead to the finding of MGUS initially. Thus, it is currently unknown whether MGUS ascertained through screening is associated with excess mortality.

1.3.5 Thrombosis

Several investigators have found an increased risk of venous thrombosis in individuals with MGUS compared to the general population.[81,82,107,108] In addition, Kristinsson et al. reported an increased risk of arterial thrombosis in 5,395 individuals with MGUS compared to matched controls without MGUS[82]. Conversely, Za et al. found that the rate of arterial and venous thrombotic events in a retrospective cohort of 1,491 MGUS patients did not appear higher than that reported in the general population, however, no control group was used in that study.[109]

In regard to MGUS isotype, individuals with IgG MGUS have in one study been shown to be less prone to develop thrombosis than other MGUS isotypes,[108] and in another study individuals with IgG or IgA MGUS had a greater risk than individuals with IgM MGUS.[82]

All previous studies on thrombosis in MGUS have been performed on clinically established cohorts, and are, as previously mentioned, at risk of bias due to comorbidities.

1.3.6 Light-chain monoclonal gammopathy of undetermined significance

Recently, a new entity called light-chain MGUS (LC-MGUS) has been described.[17,110] In LC-MGUS, there is only secretion of either kappa or lambda FLC. There is no monoclonal immunoglobulin heavy chain expression, no end-organ damage attributable to lymphoproliferative disorder, clonal bone marrow plasma cells of <10%, and urinary M-protein of <500 mg/24 hours.[17] Individuals with LC-MGUS are at an increased risk of progression to LC-MM or amyloid light-chain amyloidosis.[17,43,110]

The current definition of LC-MGUS is an abnormal FLC ratio (< 0.26 or > 1.65), and an increased level of the involved light chain of more than 19.4 mg/L in kappa FLC and more than 26.3 mg/L in lambda FLC.[17] Since FLCs are cleared from the serum by the kidneys, a decrease in renal function leads to an increase in serum FLC levels, which is why a modified, extended range of what is considered normal FLC ratio has been suggested for individuals with renal failure (0.37-3.1).[110-112] The upper and lower limits of kappa and lambda were established using the normal distribution of free light chains in 282 individuals.[113]

Currently, the information available regarding LC-MGUS is based on only two studies.[93,110] Dispenzieri et al used FLC assay, IFE, and SPEP to screen 18,357 individuals of 50 years and older in Olmsted County and found 146 cases of LC-MGUS, corresponding to a prevalence of 0.8% (Table 3).[110] The prevalence of LC-MGUS was, as in MGUS, higher in men than in women. They found the prevalence of LC-MGUS to be increasing with age up to around 80 years of age in men, after which it levelled off, whereas in women the prevalence of LC-MGUS increased consistently throughout the higher age-groups. The highest incidence was thus found among men aged 70-79 years (1.7%) and women aged 80-89 years (1.5%). The modified, renal reference range was applied but not used for the definition. Only three cases in the cohort progressed during follow-up, and all to LC-MM.

Eisele et al screened 4,702 serum samples from German men and women of the Heinz Nixdorf Recall Study, aged 45-75 years, and found 34 cases of LC-MGUS, a prevalence of 0.7% (Table 3).[93] The prevalence of LC-MGUS was higher in men than in women. During a median follow-up time of five years, none of the LC-MGUS cases progressed. The risk of progression to lymphoproliferative disease thus appears to be smaller in LC-MGUS than in MGUS.

The knowledge of LC-MGUS is thus very limited, with information on prevalence based on two studies only and little information about risk of progression.

Table 3. Prevalence of LC-MGUS

	Dispenzieri et al, 2010[110]	Eisele et al, 2012[93]
Number of participants	18,357	4,702
Number of LC-MGUS	146	34
Prevalence (total)	0.8%	0.7%
Age 40-49 years	-	0.3%
Age 50-59 years	0.5%	0.2%
Age 60-69 years	0.8%	1.0%
Age 70-79 years	1.1%	1.7%
Age 80-89 years	1.3%	-
Median age	68 years (range 50-96)	67 years (range 47-74)
Kappa LC-MGUS	108 (74%)	28 (82%)
Lambda LC-MGUS	38 (26%)	6 (18%)
Median concentration of involved FLC	176 mg/L	-
Number who progressed	3	0
Risk of progression	0.3 per 100 person-years	-

2 AIM

Overall aim

The overall aim was to increase our understanding of MGUS and LC-MGUS, with the purpose of improving the management of patients with these conditions.

Hypotheses

1) The diagnostic criteria for LC-MGUS can be improved to increase accuracy and better capture cases of clinical importance.

2) MGUS and LC-MGUS is a common condition in an elderly population and its prevalence can be quantified through screening using SPEP, IFE, and FLC analysis

3) Individuals with MGUS and LC-MGUS have inferior survival compared to individuals without MGUS

4) Autoimmune disease has a negative effect on survival in MGUS and in MM

5) Clinical follow-up of individuals with MGUS leads to better survival in MM

6) The causes of death in individuals with MGUS and LC-MGUS differ from individuals without MGUS.

7) Individuals with MGUS and LC-MGUS are at increased risk of arterial and venous thrombosis

3 PREVALENCE OF MGUS AND LIGHT-CHAIN MGUS (I)

Our aims of the study (Paper I) presented below were to quantify the prevalence of MGUS and LC-MGUS in a screened population, and to simplify and improve the diagnostic criteria for LC-MGUS.

3.1 METHODOLOGICAL CONSIDERATIONS

In order to quantify the prevalence of MGUS and LC-MGUS in the population, and to determine an adequate and useful definition of LC-MGUS, we used two population-based cohorts (see below) and screened for MGUS and LC-MGUS using SPEP, IFE (Helena Laboratories, Beaumont, Texas, USA), and FLC analysis (FREELITE, The Binding Site Ltd, Birmingham, UK). The FLC assay measures free kappa and free lambda light-chain concentrations as well as the kappa-to-lambda ratio (FLC ratio).[113] Individuals whose samples contained one or more M-protein bands on SPEP and/or IFE were considered to have MGUS, and those samples were subjected to IFE to determine the MGUS isotype, and M-protein concentration was measured. Since different lymphoproliferative disorders also can have an M-protein on SPEP, individuals with a lymphoproliferative diagnosis at study baseline were identified and excluded. In each cohort, the prevalence of MGUS was calculated in crude number and as in percent of the entire cohort, along with numbers and prevalence of different isotypes.

Renal function for all individuals in the AGES-Reykjavik Study cohort was calculated as glomerular filtration rate (GFR) in milliliters per minute per $1.73m^2$, using the Modification of Diet in Renal Disease formula, which takes into account creatinine level, age, and sex.[114] Analyses for the prevalence of LC-MGUS were performed based on the previous definition of LC-MGUS, with and without the renal reference range, and exploratory analyses using the normal distribution of kappa and lambda values.[110] The results from the different analyses were used to create new cut-off values for FLC concentrations, which were then applied to the cohorts. The prevalence of LC-MGUS, and the number of kappa and lambda LC-MGUS, was determined in each step. In the American cohort, the prevalence of LC-MGUS was estimated in whites, blacks, and Asian/Pacific Islanders separately.

Methodologically, the use of SPEP and IFE to detect MGUS is uncontroversial. However, the criteria for MGUS, according to the International Myeloma Working Group, require low prevalence of clonal plasma cells in the bone marrow and absence of end-organ damage attributable to plasma cell disease. [17] Alas, the serum analyses were performed on stored samples, and we did not have the opportunity to examine all screened patients with regard to end-organ damage or bone marrow testing. This is a limitation of the screening method used and it is not inconceivable that some of the individuals with positive SPEP and/or IFE were in fact in the early stages of an undetected lymphoproliferative disorder. FLC analysis is the suggested and the only approach to detect LC-MGUS.

3.1.1 AGES-Reykjavik Study cohort

The cohort of the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study) is based on a prospective study of 30,795 Icelandic men and women.[115] It originated as the Reykjavik Study in 1967, and was initiated by the Icelandic Heart Association. Men and women born 1907-1934 and living in Reykjavik were invited to participate in an elaborately designed examination schedule divided into six stages, spanning over almost 30 years (1967-1996). The purpose of the Reykjavik Study was to prospectively study cardiovascular disease, and to identify risk factors for cardiovascular disease. Some of the findings from the Reykjavik Study include establishing family history of myocardial infarction, erythrocyte sedimentation rate, C-reactive protein as independent risk factors for myocardial infarction, and showing a family history of lung cancer to be an independent risk factor for lung cancer.[116-119]

In 2002, individuals were randomly selected from survivors of the Reykjavik Study, and 5,764 individuals (a response rate of 75%) were re-examined for the AGES-Reykjavik Study.[115] The purpose of the AGES-Reykjavik Study was to study environmental and genetic contributions to diseases of the elderly, with special focus on four biologic systems: vascular, neurocognitive, musculoskeletal, and body composition/metabolism. The study design allows for the combination of midlife data from the Reykjavik Study and old-age data from AGES-Reykjavik, for life course study. Participants in the AGES-Reykjavik Study examination in 2002-2006 completed a questionnaire, underwent clinical examination, laboratory testing, and radiological examinations.

The AGES-Reykjavik Study cohort is longitudinal and ongoing, and information on incidence of disease, date of death, and cause of death is collected annually through hospital, nursing home, and mortality records. End of follow-up for the analyses of outcome in the present study was March 31, 2014. Study baseline was date of first visit in AGES-Reykjavik Study. To capture individuals who progressed to a lymphoproliferative disorder, we used information from the Icelandic Cancer Registry and hospital records. For individuals who progressed, individual medical records were assessed and diagnosis of lymphoproliferative disorder was validated.

3.1.2 PLCO cohort

The Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial is a large, randomized trial designed to determine if screening for these four cancers can decrease the mortality in these diseases.[120,121] The trial recruited from 1992 to 2001 around 155,000 volunteers, aged 55 to 74 years, who were randomized to attend either a cancer screening regimen, or to routine medical care. For the present study, a random sample of 5,916 individuals were selected from participants in the screening arm of the trial who had available serum and were not diagnosed with a lymphoproliferative disorder prior to study entry or during follow-up (end of follow-up January 1st, 2012). The sampling was performed within strata by sex, age at baseline, and race. No further exclusion criterion was applied. Study baseline was date of blood sample collection.

3.2 RESULTS AND DISCUSSION

From the AGES-Reykjavik cohort of 5,725 included participants, MGUS was identified, through one or several M-protein bands on SPEP, in 300 individuals, corresponding to a prevalence of 5.2% (Table 4).

Among the 5,916 individuals of the PLCO cohort, 283 cases of MGUS were identified, corresponding to a prevalence of 4.8%. The prevalence of MGUS was highest in the 70-79 years age group, and higher in men than in women ($p<0.001$), in both cohorts. The median age in the AGES-Reykjavik Study cohort was higher than in the PLCO cohort, with more individuals in the 70-79 years age group, which is likely the explanation behind the slightly higher prevalence (5.2%) in the AGES-Reykjavik Study cohort compared to the PLCO cohort (4.8%). The prevalence of MGUS is known to increase with age.[22,89,93,110] The prevalence of MGUS in our study cohorts is similar to that found in previous studies, which confirms the prevalence of MGUS to be 4-5% in a population over 60 years of age.[22,89-93]

In the AGES-Reykjavik Study cohort, 453 individuals (7.9%) had a pathological FLC ratio but normal SPEP, and 275 of these (4.8%) also had an increased concentration of the light chain involved (>19.4 mg/L kappa, or >26.3 mg/L lambda). This resulted in 264 kappa and 11 lambda cases of LC-MGUS, a kappa prevalence of 96%, according to the previous definition.[110] When applying the modified, renal reference range (0.37-3.1) to individuals with an impaired renal function (GFR <60 mL/min), this resulted in 135 individuals with LC-MGUS with a kappa prevalence of 92%. Only applying the renal reference range to individuals with severe renal failure (GFR <30 mL/min) increased the number of LC-MGUS to 249, with persisting high kappa prevalence of 96%. Similarly, applying the previous definition of LC-MGUS to the PLCO cohort, 286 cases of LC-MGUS were identified with a kappa prevalence of 95%.

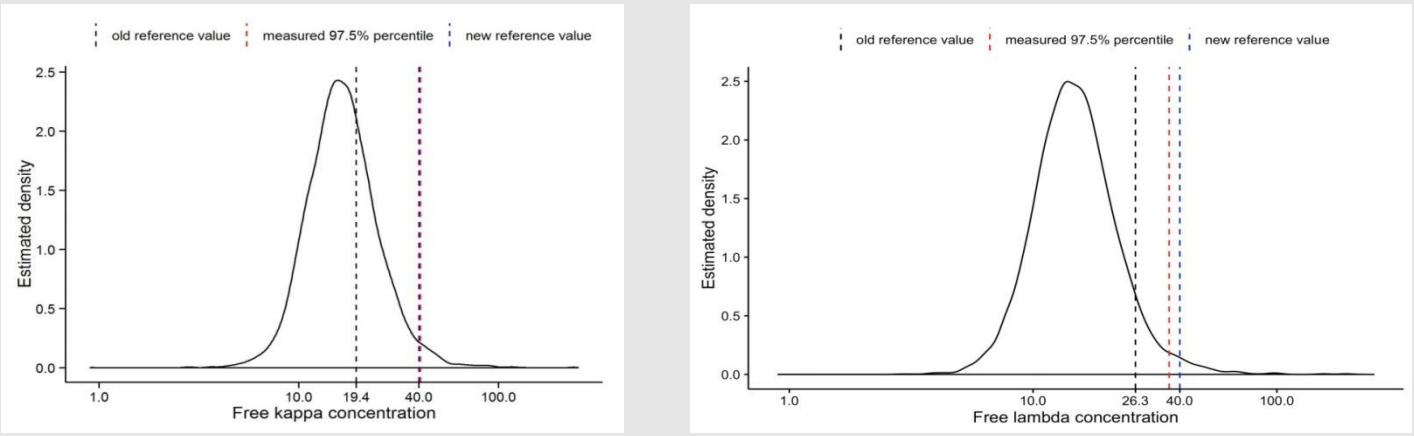
Table 4. Results from analysis of the AGES (5,725 participants) and PLCO (5,916 participants) cohorts

	AGES			PLCO		
	MGUS[a]	**LC-MGUS[b]**	**No MGUS**	**MGUS**	**LC-MGUS**	**No MGUS**
Total n[c] of cases (%)	300 (5.2)	52 (0.9)	5,373 (93.9)	283 (4.8)	57 (0.96)	5,576 (94.3)
Men, n (%)	159 (53.0)	34 (65.4)	2,226 (41.4)	201 (71.0)	41 (71.9)	3,452 (61.9)
Women, n (%)	141 (47.0)	18 (34.6)	3,147 (58.6)	82 (29.0)	16 (28.1)	2,124 (38.1)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)	72 (60-80)	71 (60-79)	69 (59-80)
Age group, n (%)						
Less than 70 years	21 (7.0)	1 (1.9)	529 (9.9)	107 (37.8)	22 (38.6)	2,876 (51.6)
70-79 years	148 (49.3)	16 (30.8)	3,039 (56.6)	173 (61.1)	35 (61.4)	2,685 (48.2)
80-89 years	120 (40.0)	33 (63.5)	1,679 (31.2)	3 (1.1)	0 (0)	15 (0.3)
90 years and older	11 (3.7)	2 (3.8)	126 (2.3)	0 (0)	0 (0)	0 (0)
MGUS isotype, n (%)						
IgG	159 (53.0)	N.A.[d]	N.A.	179 (63.3)	N.A.	N.A.
IgA	27 (9.0)	N.A.	N.A.	32 (11.3)	N.A.	N.A.
IgM	81 (27.0)	N.A.	N.A.	55 (19.4)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.	0 (0)	N.A.	N.A.
Biclonal	32 (10.7)	N.A.	N.A.	17 (6.0)	N.A.	N.A.
M-protein concentration, n (%)[e]						
>15.0 g/L	17 (5.7)	N.A.	N.A.	9 (3.2)	N.A.	N.A.
<15.0 g/L	147 (49.0)	N.A.	N.A.	207 (73.1)	N.A.	N.A.
missing	136 (45.3)	N.A.	N.A.	67 (23.7)	N.A.	N.A.
FLC[f] ratio						
0.26-1.65	168 (56.0)	0 (0.0)	4972 (92.5)	176 (62.2)	0 (0)	5,347 (95.9)
<0.26 / >1.65	132 (44.0)	52 (100.0)	401 (7.5)	107 (37.8)	57 (100)	229 (4.1)
Race						
White	N.A.	N.A.	N.A.	185 (65.4)	27 (47.4)	3,787 (67.9)
Black	N.A.	N.A.	N.A.	70 (24.7)	28 (49.1)	857 (15.4)
Asian/Pacific Islander	N.A.	N.A.	N.A.	28 (9.9)	2 (3.5)	932 (16.7)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]N.A.: not applicable, [e]available for 164 subjects in AGES, for 216 subjects in PLCO, [f]FLC: free light chain.

Considering the surprisingly high prevalence of LC-MGUS and the kappa-biased results, we performed additional analyses using the normal distribution and the log-transformed values of kappa and lambda. We used the entire AGES-Reykjavik Study and PLCO cohorts, excluding the 300 and 283 individuals with MGUS, respectively, and determined the 2.5th and the 97.5th percentiles of kappa concentration, lambda concentration, and FLC-ratio (Figure 4). These were found to be very similar in the two cohorts: 7.7-40.6 mg/L and 7.3-37.3 mg/L for kappa, 7.2-36.3 mg/L and 6.8-32.3 mg/L for lambda, and 0.6-2.0 and 0.6-1.8 for the FLC-ratio, in the AGES-Reykjavik Study and the PLCO cohorts, respectively.

a)



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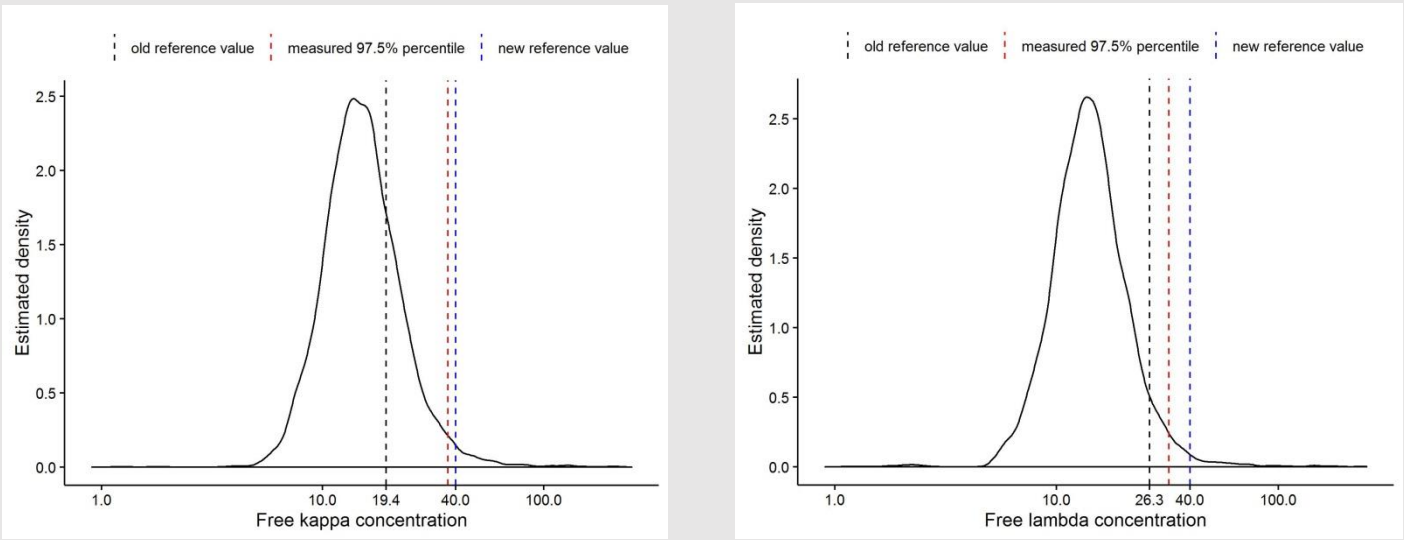


Figure 4. The distribution of serum free kappa and lambda concentrations among individuals without M-protein on serum protein electrophoresis (the 97.5th percentile, prior cut-offs for LC-MGUS, and 40 mg/L are marked) in a) the AGES-Reykjavik Study and b) the PLCO Study. The x axis is on a log transformed scale with base 10.

With the purpose of evaluating the requirement for a renal reference range, which has previously been suggested, we examined the 2.5th and the 97.5th percentiles of FLC concentration and FLC-ratio among individuals with different degrees of impaired renal function in the AGES-Reykjavik cohort. In the 2,131 individuals with moderately impaired renal function (GFR ≥30 or ≤60 mL/min), the percentiles were 0.9-43.6 mg/L for kappa, 8.0-38.1 mg/L for lambda, and 0.7-2.0 for FLC-ratio. In the 105 individuals with severely impaired renal function (GFR <30 mL/min), the percentiles were 14.8-112.6 mg/L for kappa, 13.0-160.3 mg/L for lambda, and 0.5-2.2 for FLC-ratio. Concluding that the 2.5th and the 97.5th percentiles of FLC concentration and FLC-ratio in moderately impaired renal function were very similar to the values of individuals with normal renal function, and the 2.5th and 97.5th percentiles of FLC-ratio was essentially unchanged even in severe renal function, we decided that the suggested renal reference range (0.37-3.1) is of little use in the definition of LC-MGUS. Similarly, Dispenzieri et al. also concluded that the normal reference range allowed for the greatest consistency.[110]

Finally, we evaluated the effect of using the 97.5th percentile as a cut-off for normal values of the involved light-chain, instead of the previously suggested levels (19.4 mg/L for kappa and 26.3 mg/L for lambda). For simplicity, since the 97.5th percentile was close to 40 mg/L in both cohorts, this was chosen as the upper limit for both kappa and lambda. We then used a definition of LC-MGUS as a pathological FLC-ratio (unchanged from the previously suggested normal range of 0.26-1.65), regardless of renal function, in combination with an increased concentration of more than 40 mg/L of the light-chain involved. This resulted in 52 LC-MGUS cases in the AGES-Reykjavik Study cohort, a prevalence of LC-MGUS of 0.9%, and 57 cases in the PLCO cohort, a prevalence of 1.0% (Table 4). The kappa prevalence was 79% and 74% in the two cohorts, respectively.

The LC-MGUS prevalence in our study of 0.9-1.0% is similar to the prevalence found in the only two studies that have previously been performed.[93,110] The prevalence of LC-MGUS in the PLCO cohort was highest among blacks (2.9%), and lowest among Asian/Pacific Islanders (0.2%), compared to white Caucasians (0.7%), a similar race distribution to that previously described for MGUS.[22,90,92]

MGUS and LC-MGUS are of clinical importance due to the risk of malignant transformation. An accurate definition of LC-MGUS is thus of great importance, and needs to be sensitive enough to identify clinically relevant patients, who are at risk of progression and could benefit from clinical follow-up, yet specific enough not to include too many individuals who will never develop a malignant disease and have nothing to gain from follow-up. To assess the sensitivity of our revised definition, we examined the incidence of progression to lymphoproliferative disorder in the AGES-Reykjavik Study cohort. During follow-up time, three individuals who fulfilled both the previous and our revised criteria progressed: one to LC-MM, one to amyloid light-chain amyloidosis, and one to diffuse large B-cell lymphoma. One individual developed diffuse large B-cell lymphoma three years after baseline, and had an elevated FLC-ratio at baseline, but had an impaired renal function (GFR = 50 mL/min) and did thus not fulfill either the previous or the revised criteria for LC-MGUS. In summary, compared to the prior definition of LC-MGUS, the new definition that we are suggesting did not miss any additional cases of LC-MGUS.

We believe our study lays a strong foundation for a revised definition of LC-MGUS, based on normal distributions just like the original suggestion, but on a larger and more heterogeneous population (Table 5).[113] Our new definition of LC-MGUS differs from the

previously suggested foremost in precision, but also in simplicity; it is easier to apply since the cut-off is the same for both kappa and lambda (40 mg/L), and does not depend on renal function.[93,110,113] Applying our revised criteria on our two cohorts, considerably fewer individuals are diagnosed with LC-MGUS than would have been the case with the previous definition, and although it still successfully identifies the individuals who will later develop a clinically relevant lymphoproliferative disorder.

Table 5. New revised criteria for LC-MGUS[a]

Abnormal free light-chain ratio (< 0.26 or > 1.65)[b,c]
Elevated involved light chain (40 mg/L or higher)
No immunoglobulin heavy chain M-spike by SPEP/IFE[d]
No evidence of end-organ damage that can be attributed to lymphoproliferative disorder

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]values based on the serum Freelite assay (The Binding Site Group, Birmingham, UK), [c]regardless of renal function, [d]SPEP: serum protein electrophoresis and IFE: immunofixation.

MGUS and LC-MGUS are challenging conditions to study, due to their asymptomatic nature. A screened cohort study such as the present one is the only way to gain an accurate estimate of the true prevalence in the population. The major strengths of our study is its size, based on more than 11,000 participants, the heterogeneity of the cohorts; with different age groups, different ethnicities, and both genders represented, and the screening efforts underlying the findings of MGUS and LC-MGUS. Limitations include, as previously mentioned, the inability to perform bone marrow samples and clinical assessment on all individuals, lack of information on renal function and on progression in the PLCO cohort, and a limited number of patients who progressed in the AGES-Reykjavik Study cohort during follow-up which makes it difficult to assess the risk of progression. Furthermore, participants in the AGES-Reykjavik Study were selected survivors from the original Reykjavik Study cohort, and are likely to be healthier than the general population.

In conclusion, in this large international investigation, based on two independent, screened cohorts, we have determined that among the elderly, the prevalence of MGUS and LC-MGUS is 4.8-5.2% and 0.9-1.0%, respectively. We suggest a revised definition of LC-MGUS that captures the clinically relevant individuals and can be used regardless of renal function. The present study adds significantly to the field considering that until date, knowledge of FLC levels in general and of LC-MGUS in particular has been based on few studies, containing small numbers of individuals with LC-MGUS, all studies restricted to Caucasians.[93,110,113] The major clinical implication of the revised definition is that considerably fewer individuals will be diagnosed with LC-MGUS, which will decrease unnecessary health-care costs as well as reduce the burden of anxiety among affected patients. Ideally, our proposed definition would be replicated in a larger cohort study with even longer follow-up, but in the meantime we recommend the diagnostic criteria for LC-MGUS to be revised according to our suggestions.

4 NATURAL COURSE AND SURVIVAL (II, III, IV)

In our investigations on natural course and survival, our aims were to compare the survival of individuals with MGUS and LC-MGUS to individuals without MGUS, to determine whether autoimmune disease has a negative effect on survival in MGUS and in MM, and to establish whether clinical follow-up of individuals with MGUS leads to better survival in MM.

4.1 METHODOLOGICAL CONSIDERATIONS

In order to study the natural course and survival of MGUS and LC-MGUS we conducted three population-based studies, using an Icelandic cohort of individuals with MGUS and LC-MGUS, and a Swedish cohort of MGUS patients.

The Icelandic cohort of the AGES-Reykjavik Study was described previously. To examine survival in MGUS and in LC-MGUS, we used the entire cohort, including the 300 individuals with MGUS and 52 individuals with LC-MGUS that we had detected through screening, using the revised definition of LC-MGUS. The participants in AGES-Reykjavik Study are followed prospectively, with information on incidence of disease and date of death collected annually through medical records and registers. End of follow-up for the analysis of survival was March 2014. We excluded individuals who had a lymphoproliferative disorder as cause of death without previous diagnosis of lymphoproliferative disorder, and individuals with shorter follow-up time than ten days.

For the analysis of survival in MM and in MGUS after autoimmune disease, and survival in MM depending on previously known MGUS, we used Swedish cohorts of MM patients and MGUS patients. In Sweden, all physicians are obliged to report each case of cancer to the nationwide Swedish Cancer Register. We identified 8,367 MM patients diagnosed from 2000 to 2013 in the Swedish Cancer Register.[122] We also used 18,768 patients with MGUS, diagnosed from 1988 to 2013, identified from an MGUS cohort established through a national network of hematologists and oncologists, and the Swedish Inpatient and Outpatient Registers.[123] For each patient, four population-based control subjects were randomly sampled, and information on autoimmune disease in patient and controls were collected from the Swedish Inpatient Register.[30,124]

For the analysis of survival after progression to MM, depending on known MGUS status before diagnosis of MM, we used again the Swedish Cancer Register to identify 14,404 MM patients diagnosed from 1976 to 2005. We cross-linked them against the MGUS cohort mentioned previously, to determine who among the MM patients had a previously known, and thus with great likelihood clinically followed, MGUS. Survival was compared, from time of MM diagnosis, between patients with and without prior knowledge of MGUS. Information on date and cause of death was gathered from the Swedish Cause of Death Register. The completeness and diagnostic accuracy are high for the Swedish registers used.[125,126]

In all of the analyses, difference in survival was estimated using Kaplan Meier model, the log rank test, and Cox regression model.[127-129] Adjustments were made for potential confounders such as age and gender, as well as M-protein isotype and M-protein concentration, when this was available. For analyses using Cox regression model, the proportional hazards assumption was tested using plotting of the Schoenfeld residuals, a formal statistical test, and through introducing time-varying covariates into the model. The

correlation between continuous variables and categorical variables was assessed using Pearson product moment correlation coefficient and chi-squared tests, respectively. Statistical results were considered significant at $p < 0.05$.

We were able to perform these large cohort studies owing to the high quality Swedish registers and to access to the Icelandic AGES-Reykjavik Study cohort. Despite the fact that the analysis was performed retrospectively, the design is prospective in the sense that individuals in all mentioned cohorts were followed and information on outcomes was collected in the forward directionality from time of diagnosis/blood sample collection. The cohort study design is an excellent methodology to study rare exposures such as MGUS or LC-MGUS, and has the further advantage of excluding recall bias, since ascertainment of exposure status was performed through registers and, in the case of the AGES-Reykjavik Study cohort, through screening. Additionally, the prospective cohort study design allows for speculations of causality among detected associations in a way that cross-sectional studies do not. One methodological limitation is that the Swedish MGUS cohort was not established through screening, but through a national network and through registers.

4.2 RESULTS AND DISCUSSION

When studying the survival of individuals in the AGES-Reykjavik Study cohort (Paper II), we found that the five-year survival rates for individuals with MGUS, LC-MGUS, and no MGUS were 76% (95% CI 0.71-0.81), 52% (0.4-0.6), and 84% (0.82-0.84), respectively (Figure 5).

When adjusted for age and sex, individuals with MGUS and LC-MGUS had a significantly higher risk of death (HR = 1.2, 1.04-1.4, and HR = 1.6, 1.2-2.3, respectively) compared to individuals without MGUS during a median follow-up time of 9.7 years. The increased risk of death persisted after exclusion of individuals who progressed to lymphoproliferative disease, although the increased risk was then statistically significant only for LC-MGUS.

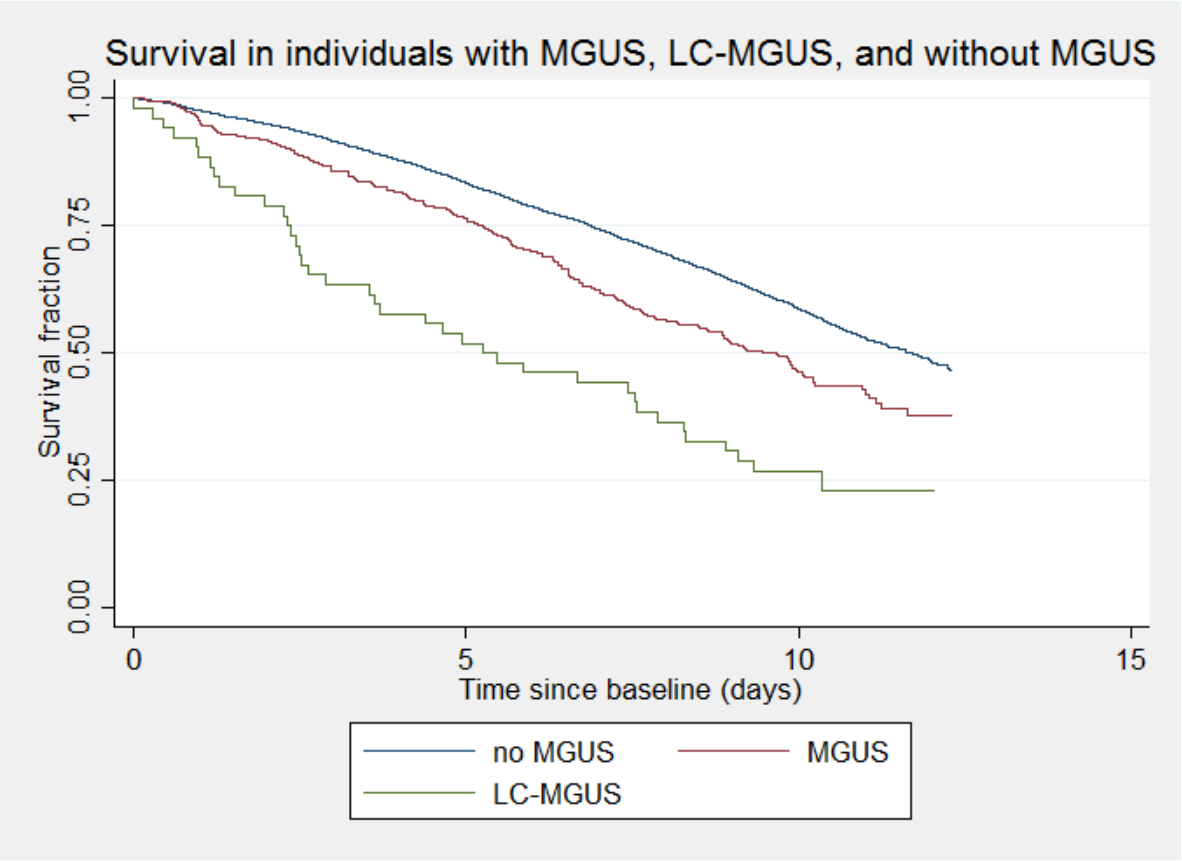


Figure 5. Survival in individuals with MGUS and LC-MGUS compared to individuals without MGUS.

We investigated the effects of different MGUS isotypes, M-protein concentration, and FLC ratio, and found that individuals with MGUS isotype A had a significantly increased risk of death compared to individuals with other isotypes (HR = 1.8, 1.1-2.9). Moreover, a high M-protein concentration or an abnormal FLC ratio did not statistically increase the risk of death when adjusted for age and sex.

The finding that individuals with LC-MGUS and MGUS have an increased risk of death compared to individuals without MGUS is interesting, since the individuals under study were diagnosed with MGUS and LC-MGUS through screening, and not clinically detected during workup for some other condition. This is, to our knowledge, the first investigation where results show inferior survival in MGUS in a screened cohort. Our results are in line with findings from previous studies from non-screened cohorts, but are unlikely to be explained by the comorbidities inevitably present in clinical cohorts of MGUS patients.[98,100,105,106] Individuals with LC-MGUS were older at baseline (82 years) than individuals with MGUS (78 years) or without MGUS (76 years), but since age was adjusted for in the analyses, it is unlikely to explain the inferior survival of individuals with LC-MGUS.

With regard to the increased risk of death in individuals with LC-MGUS, it is not necessarily the monoclonal FLCs that cause the mortality, but it could also be due to the overload of FLCs regardless of clonality. It has been shown that in the general population, non-clonal FLCs predict decreased overall survival, independently of renal function, sex, and

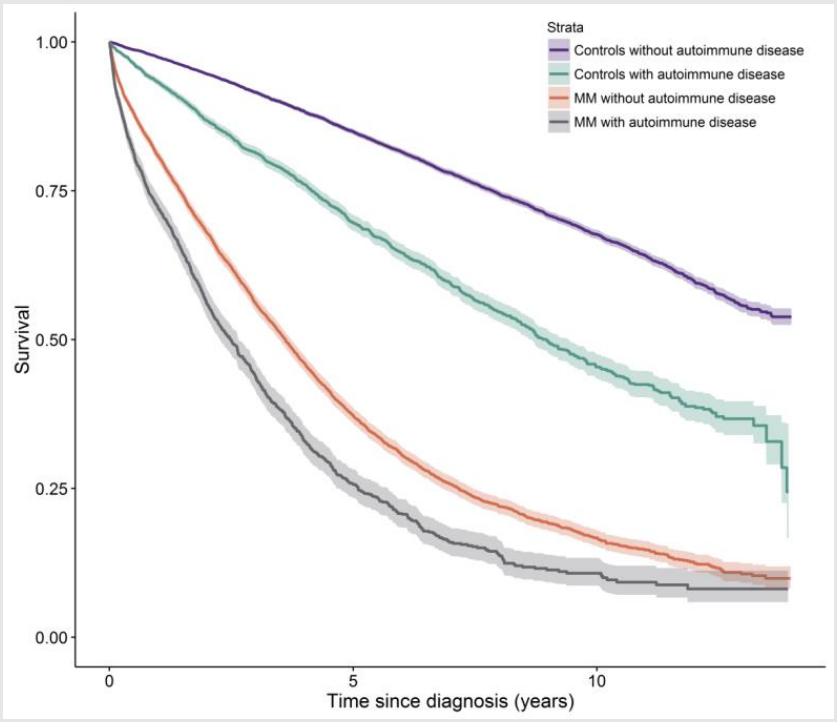
age.[130] An increase in polyclonal FLCs is seen in autoimmune diseases such as rheumatoid arthritis and Sjögren's syndrome[131]

One reason for the increased risk of death in individuals with MGUS and LC-MGUS could of course be progression to a lymphoproliferative disorder, which either goes clinically unnoticed, or for some reason is not noted in medical records or registers. When individuals who developed a lymphoproliferative disorder during follow-up were excluded from the analyses, the risk of death remained increased for LC-MGUS (HR = 1.5, 1.1-2.1) and MGUS (HR = 1.1, 0.9-1.3), although the risk estimate was no longer statistically significant for the latter. Since the individuals in the AGES-Reykjavik Study cohort were not planned to be re-examined in the study after baseline, with the goal of detecting early signs of malignancy, and individual medical records were not routinely evaluated, cases of undetected progression to lymphoproliferative disorder in our cohort cannot be entirely excluded. However, several factors speak against this, including the rareness of lymphoproliferative disorders, the completeness of the registries used, and the Icelandic health care system which is well-functioning and provides affordable health care to the entire population.

We believe that the inferior survival noticed among individuals with MGUS and LC-MGUS in our cohort is indeed a true reflection of an increased risk of death in these disorders, and is not caused by malignant progression. The increased risk could be due to an underlying genetic susceptibility for both plasma cell disorders and other conditions, or to the overproduction of light chains causing previously undetected organ damage.

In the Swedish cohorts, a personal history of autoimmune disease was found in 16% of MM patients and in 21% of MGUS patients, compared to 13% in MM controls and 12% in MGUS controls, respectively (Paper III). In individuals with both MM (HR = 1.2, 95% CI 1.2-1.3) and MGUS (HR = 1.4, 1.3-1.4), a decreased survival was associated with a personal history of autoimmune disease (Figure 6). In particular, a history of ulcerative colitis had a stronger negative impact on survival in MM than in controls. The effect of autoimmunity on survival was not different between individuals with different MGUS isotypes or M-protein concentration, or between men and women.

a)



b)

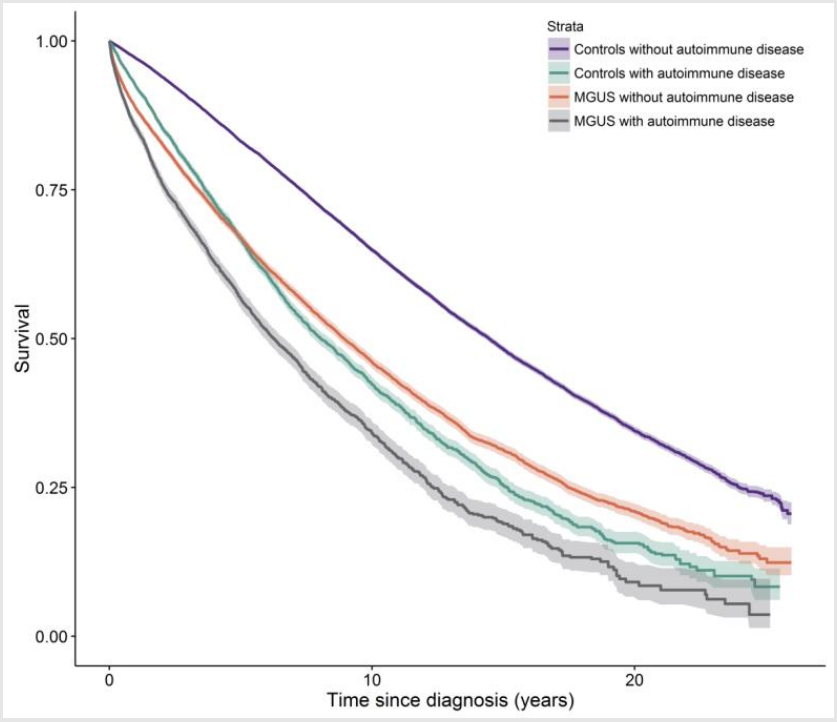


Figure 6. Survival in MM (a) and in MGUS (b) with and without a personal history of autoimmune disease, compared to controls.

The observation that a personal history of autoimmune disease is associated with an increased risk of death in both individuals with MM and MGUS could also be due to shared genetic susceptibility for plasma cell disorders and autoimmune disease, or to cumulative comorbidity in the individual. We know that autoimmunity is associated with an increased risk of developing MGUS and MM, and possibly, autoimmunity triggers a more severe form of MM – but this could not account for the increased risk of death detected in individuals with MGUS who do not progress.[21,30]

We studied survival among 14,798 MM patients, whereof 394 had previously been diagnosed with MGUS (Paper IV). We found that patients with MM with prior knowledge of MGUS had significantly better overall survival (median survival 2.8 years) than MM patients without prior knowledge of MGUS (median survival 2.1 years), even though the former had more comorbidities ($p<0.001$) (Figure 7). The results were similar for cause-specific survival, where the risk of dying from MM was lower (HR = 0.75, 0.6-0.9) for MM patients with a prior knowledge of MGUS compared to those without prior knowledge of MGUS.

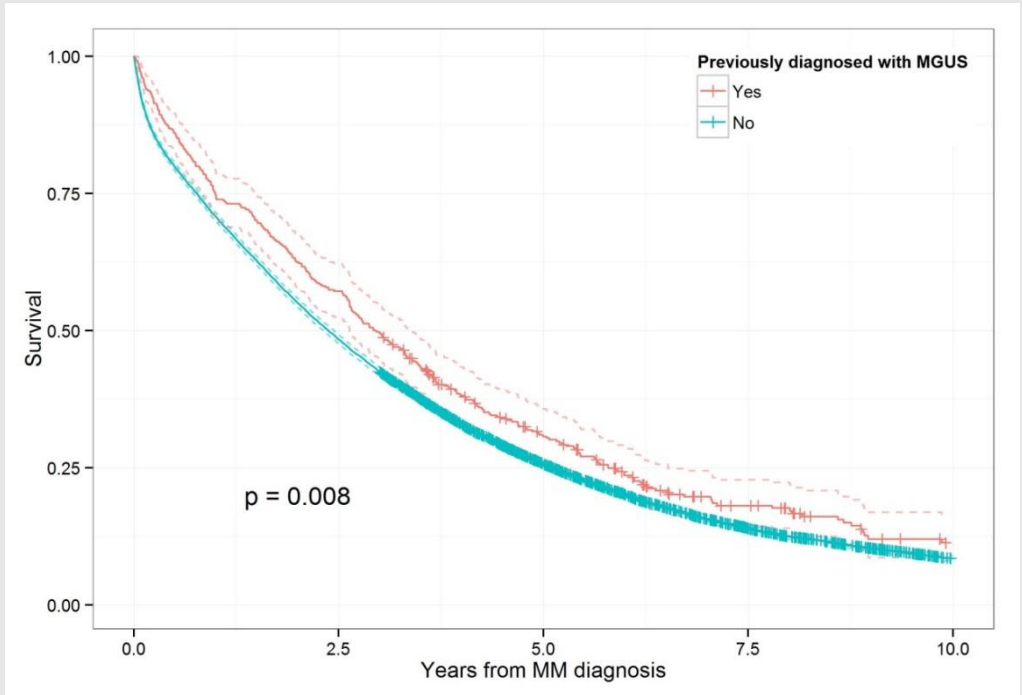


Figure 7. Survival in MM patients with and without prior knowledge of MGUS.

Furthermore, we found that among MM patients with prior knowledge of MGUS, a low M-protein concentration at MGUS diagnosis was predictive of inferior survival in MM (HR = 1.86, 1.1-3.0), and the median time from MGUS to MM diagnosis was shorter for those with a high M-protein concentration. There was no difference in survival between different MGUS isotypes.

In the cohort under study, the risk of death was 14% lower in MM patients with a prior diagnosis of MGUS, which confirms results from a recent publication demonstrating a 13% better overall survival.[132] This makes a strong case for the benefit of clinical follow-up of individuals with MGUS. Curiously, a low M-protein concentration predicted inferior survival in MM, which is contradictory to the current belief that a high M-protein concentration increases the risk of progression from MGUS to MM.[101] One reason for this could be current guidelines recommending less frequent monitoring of low-risk MGUS patients, which could lead to a delayed diagnosis of progression to a lymphoproliferative disorder.[101] If this were the case, it would speak against current guidelines and argue for regular and prevalent follow-up of individuals with MGUS regardless of risk profile.

One reason behind the detected difference in survival could be that the MM patients with a prior knowledge of MGUS do not in fact *survive* longer, but rather are diagnosed earlier, and therefore *followed* (from detection of disease until end of follow-up) longer, a so called lead-time bias. If this were the case, MM patients with a prior knowledge of MGUS would be diagnosed at an earlier age. To investigate this, we compared median age at diagnosis in the two groups, which was similar, and furthermore we compared days between diagnosis of MGUS and diagnosis of MM, and age at diagnosis of MM, and the correlation was low (0.03 by Pearson's product moment correlation coefficient).

We found that the MM patients with prior knowledge of MGUS had higher prevalence of comorbidities than did MM patients without prior knowledge of MGUS, which confirms the previously stated assumption that clinically detected MGUS is often the result of workup for an unrelated disease, and underlines the need for studies based on screened, population-based cohorts. It also suggests that in individuals with MGUS detected through screening, the benefit of clinical follow-up would be even greater.

In summary, we found that individuals with LC-MGUS and MGUS have a higher risk of death than individuals without MGUS, that a history of autoimmune disease predicts a decreased survival in both MM and MGUS, and that prior knowledge of MGUS is associated with an improved survival in MM.

The major strength of our studies on the natural course of MGUS and LC-MGUS, and on the survival in MGUS and in MM, is the cohort study design, and in particular the screened cohort design of the studies on the participants of the AGES-Reykjavik Study. Furthermore, the cohorts under study were all large and population-based, and exposures such as prior knowledge of MGUS, a history of autoimmune disease, and other comorbidities, were established through registers, thus eliminating recall bias on behalf of the participants. Limitations include the inability to validate individual medical records as well as absence of information on potentially important confounders in the studies on MM patients, such as treatment and risk stratification score.

Our findings are of clinical importance, since they suggest that greater attention should be paid to comorbidity in MGUS and MM, and that follow-up of MGUS patients is important in order for early detection and treatment of malignant disease. It would be interesting to further characterize, in detail, the comorbidities and biomarkers in a large cohort of individuals with MGUS and LC-MGUS, to determine whether concurrent conditions, autoimmune disease especially, or risk factors can explain the inferior survival demonstrated in these studies.

5 CAUSES OF DEATH AND COMORBIDITIES (II, V)

We aimed to study the causes of death of individuals with MGUS and LC-MGUS, and compare them to individuals without MGUS, and to examine the risk of arterial and venous thrombosis in individuals with MGUS and LC-MGUS.

5.1 METHODOLOGICAL CONSIDERATIONS

We conducted a cohort study using the participants of the AGES-Reykjavik Study, which has been described previously (Table 6).

Table 6. Characteristics of the study participants

	MGUS[a]	LC-MGUS[b]	No MGUS
Total n[c] of cases (%)	297 (5.2)	52 (0.9)	5367 (93.9)
Men, n (%)	158 (53.2)	34 (65.4)	2222 (41.4)
Women, n (%)	139 (46.8)	18 (34.6)	3145 (58.6)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)
Age group, n (%)			
Less than 70 years	21 (7.1)	1 (1.9)	528 (9.8)
70-79 years	146 (49.2)	16 (30.8)	3037 (56.6)
80-89 years	119 (40.0)	33 (63.5)	1677 (31.2)
90 years and older	11 (3.7)	2 (3.8)	125 (2.3)
MGUS isotype, n (%)			
IgG	158 (53.2)	N.A.	N.A.[d]
IgA	27 (9.1)	N.A.	N.A.
IgM	79 (26.6)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.
Biclonal	32 (10.8)	N.A.	N.A.
M-protein concentration, n			
>15.0 g/L	17 (5.7)	N.A.	N.A.
<15.0 g/L	145 (48.8)	N.A.	N.A.
Information missing	135 (45.5)	N.A.	N.A.
FLC[e] ratio			
0.26-1.65	167 (56.2)	0 (0.0)	N.A.
<0.26 / >1.65	130 (43.8)	52 (100.0)	N.A.

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]NA: not applicable, [e]FLC: free light chain.

To study causes of death, we used a previously described categorization, and estimated mortality rate ratios for each cause of death using Cox proportional hazards model.[55,127] The cause-specific mortality estimates show the actual risk in the cohort of death from the causes

investigated. However, in a cohort of elderly individuals such as the one at hand, the participants are subject to several potential events that could impede the occurrence of other events. One example is, when looking at risk of death from malignant disease, one individual who dies from heart disease at five years of follow-up might have died from malignant disease at eight years of follow-up, if they had not been subjected to this so called competing event. If we are interested to know the real-world risk of malignant disease, for example, then we must take into account that some participants will, due to other causes of death, not be under risk to experience this event.

One approach to cause-specific hazards is to model the cause-specific hazard of each event or category of events separately, using a standard Cox regression model, and treat other (competing) events as censored observations. Another approach is Fine and Gray's extension of that method, which models the cumulative incidence function.[133] The cumulative incidence function is the probability sub-distribution function of failure from a specific cause.[134] Each of these methods can provide useful insights about the variables in the model. We were mostly interested in the pure effects of MGUS and LC-MGUS on the different causes of death, which are most easily visualized through the cause-specific hazard model. However, in real world scenarios, patients in particular will be interested to know what their actual risk is, considering all other causes that might play a role. We therefore chose to use both models in our analyses on cause of death.

To examine the risk of thrombosis, we had access to incidence of venous and arterial thrombosis in all subjects, both as first occurrence in the health care records used, and as cause of death. Information on incidence of disease had been collected for all participants from nine years before study baseline, and all through follow-up with a median follow-up time of 8.8 years (Figure 7). However, due to data limitations, we only had access to the first incidence of thrombosis for each individual. This was indeed our outcome of interest, although, as a consequence, all individuals who had a history of thrombosis before study baseline were censored from experiencing an event during follow-up, unless that event was cause of death (we had access to cause of death for all individuals).

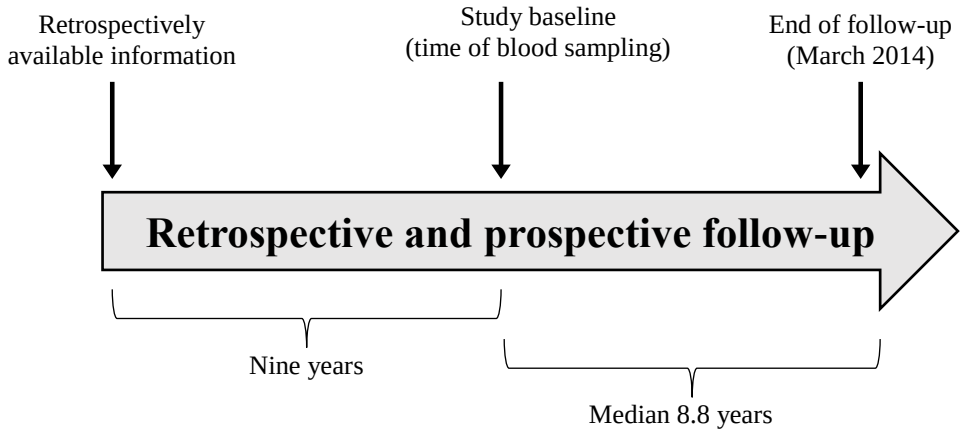


Figure 7. Study design of the AGES-Reykjavik Study cohort.

We aimed to work around this data limitation in two ways. Firstly, we did a cross-sectional analysis of study baseline information of the participants. Through logistic regression, we evaluated the association of a history of thrombosis, as assessed by a diagnosis of thrombosis from health care records as well as self-reported in the questionnaire, and MGUS status, and adjusted for age and sex as well as risk factors for venous and arterial thrombosis, respectively. Secondly, we used a Cox regression model to estimate the risk of incidence of thrombosis during follow-up. When assessing the risk of venous thrombosis (defined as pulmonary embolism, deep vein thrombosis, or other venous thrombosis), individuals with a history of venous thrombosis at baseline were excluded, and adjustments were made for comorbidity with obesity and cancer.[71,72,135] For the risk of arterial thrombosis (defined as acute coronary syndrome, ischemic stroke, or arterial embolism), individuals with a history of arterial thrombosis at baseline were excluded, and adjustments were made for known risk factors diabetes mellitus type II, hypertension, smoking, family history of arterial thrombosis, and serum cholesterol level.[136-141]

An alternative method would have been to move study baseline nine years back, and under the assumption that all individuals with MGUS or LC-MGUS detected through screening in 2002-2004 also had this condition nine years prior, to perform a retrospective prospective analysis using all individuals in the cohort. However, we chose to avoid this methodology, considering that even though MGUS is known to be present for a long time, it is impossible for us to know when it appeared in these individuals. Furthermore, such a study design would introduce an immortal time bias that would limit the generalizability of the results.

5.2 RESULTS AND DISCUSSION

In the analysis of causes of death (Paper II), we found that individuals with MGUS and LC-MGUS had an increased risk of death from the categories any cancer (HR = 1.7, 1.3-2.3, and HR = 2.3, 1.2-4.3, respectively) and any heart disease (HR = 1.4, 1.1-1.8, and HR = 1.8, 1.1-3.0), compared to individuals without MGUS. We noted with interest that the estimates were higher for LC-MGUS than for MGUS, even though the CIs were overlapping. Furthermore, individuals with LC-MGUS also had an increased risk of death from myeloid malignancy, amyloidosis, biliary/pancreatic disease, and psychiatric illness, compared to individuals without MGUS; although very few deaths occurred in these groups (Table 7).

The elevated risk of death from cancer was unsurprising to us, seeing as patients who progress from MGUS to lymphoproliferative disorders are likely to die from their malignant disease. Since we were interested in the survival patterns and causes of death among individuals with MGUS and LC-MGUS who did not undergo malignant transformation, the 56 subjects in the cohort who progressed to a lymphoproliferative disorder during follow-up were excluded.

Interestingly, the risk of death from any cancer (HR = 2.1, 1.1-4.1) and from the category any heart disease (HR = 1.8, 1.01-3.0) was still increased for LC-MGUS. The risks for MGUS were no longer statistically significant (any cancer HR = 1.3, 0.9-1.8, any heart disease HR = 1.3, 0.99-1.7). The category any heart disease comprised two subgroups; ischemic heart disease and other heart disease. Trying to characterize these risks further, we

looked at specific conditions in each category; a limited analysis due to very few deaths from each condition. We did however see that the risk of conditions in the subgroup other heart disease accounted for the increased risk in both LC-MGUS (other heart disease: HR = 2.5, 1.1-5.8) and MGUS (HR = 1.8, 1.1-2.7). The subgroup other heart disease included conditions such as rheumatic heart disease, cardiac valve disease, hypertension, cardiac failure, and different arrhythmias of the heart. Our analyses on competing risks did not substantially change the estimates.

Table 7. Risk of selected causes of death in MGUS and LC-MGUS, compared to without MGUS.

Cause of death	MGUS[a]			LC-MGUS[b]			No MGUS
	N.	HR[c]*	95% CI[d]	N.	HR*	95% CI	No.
Any cancer	51	1.7	1.3-2.3	10	2.3	1.2-4.3	552
Any hematologic malignancy	16	11.2	6.0-20.8	2	10.7	2.5-45.7	28
Multiple myeloma	11	∞	N.A.[e]	1	∞	N.A.	0
Waldenström macroglobulinemia	2	∞	N.A.	0	-	-	0
Other LD[f]	2	4.1	0.9-18.8	0	-	-	10
Myeloid malignancy	0	-	-	1	11.9	1.5-96.3	11
Any solid tumor	35	1.2	0.9-1.7	8	1.9	0.9-3.8	524
Amyloidosis	0	-	-	1	104.4	6.0-1826.7	1
Heart disease	61	1.4	1.1-1.8	15	1.8	1.1-3.0	745
Ischemic heart disease	37	1.1	0.8-1.5	9	1.3	0.7-2.5	510
Other heart disease	24	1.8	1.2-2.8	6	2.5	1.1-5.8	235
Psychiatric illnesses	3	0.6	0.2-1.8	3	3.3	1.1-10.7	97

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]HR: hazard ratio, [d]CI: confidence interval, [e]N.A.: not applicable, [f]LD: lymhoproliferative disorder. *Estimates are adjusted for age and sex.

The finding of an increased risk of death from heart disease is in line with findings in one previous study on causes of death in MGUS.[105] Our finding that the increased risk of death from heart disease is particularly prominent in individuals with LC-MGUS is especially interesting, and point at an increased risk of cardiovascular disease in individuals with LC-MGUS. This could be due to an undetected genetic, or environmental, susceptibility for both LC-MGUS and diseases of the heart or vascular system, or a common pathogenesis, or that one condition predisposes for the other. The association we found is no ground for conclusions on causality, but one might speculate that the FLC in individuals with LC-MGUS are somehow involved in the pathogenesis of cardiovascular disease. If this is the case, it is not necessarily the monoclonality in itself that is the underlying cause, but it could also be the overload of FLC that causes disease. It has been shown that elevated levels of polyclonal FLC are associated with increased mortality and, furthermore, predicts cardiovascular events in patients with diabetes.[142,143] A common etiology, or a strong connection between inflammation and atherosclerosis, could be one underlying

explanation.[144] Naturally, another explanation for the observed association could be that the individuals with LC-MGUS in our cohort suffer from undetected amyloidosis, which is causing both monoclonal FLC and heart disease.

At study baseline, a history of thrombosis was present in 30 (10.1%) individuals with MGUS, in 13 (25.0%) individuals with LC-MGUS, and in 642 (12.0%) of individuals without MGUS (Paper V). Arterial thrombosis was more common than venous thrombosis in all groups. The risk of having had an arterial thrombosis at baseline was significantly increased for individuals with LC-MGUS (OR = 2.5, 1.3-4.9), and remained increased in a model adjusted for age and sex (OR = 2.0, 1.03-3.8), but not when additional risk factors were added to the model (OR = 1.9, 0.93-3.8), which could be interpreted as a power issue (Table 8).

Similarly, during follow-up, the risk of experiencing an arterial thrombosis was almost doubled (HR = 1.9, 1.1-3.2) for individuals with LC-MGUS, compared to individuals without MGUS. When adjusting for age, sex, and risk factors for arterial thrombosis, the risk estimate was not statistically significant (HR = 1.3, 0.7-1.3).

No increased risk of venous thrombosis was found in individuals with MGUS or with LC-MGUS, compared to individuals without MGUS, in the retrospective or the prospective analysis.

Table 8. A history of thrombosis at baseline in individuals with LC-MGUS, compared to individuals without MGUS.

	LC-MGUS[a]				No MGUS[b]
	N.[c]	OR[d] (95% CI[e])			N.
		Crude	Adjusted for age and sex	Multivariate analysis[†]	
Any thrombosis	13 (25.0%)	2.5 (1.3-4.6)	1.9 (1.00-3.6)	1.9 (0.94-3.7)	642 (12.0%)
Arterial thrombosis	12 (23.1%)	2.5 (1.3-4.9)	2.0 (1.03-3.8)	1.9 (0.93-3.8)	565 (10.5%)
Venous thrombosis	2 (3.9%)	2.5 (0.6-10.5)	2.0 (0.5-8.5)	2.0 (0.5-8.5)	84 (1.6%)

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]MGUS: monoclonal gammopathy of undetermined significance, [c]N.: number of individuals, [d]OR: odds ratio, [e]CI: confidence interval.
[†]Results adjusted for age, sex, smoking, hypertension, cholesterol, diabetes mellitus type II, and family history of arterial thrombosis, and age, sex, body mass index, previous or current cancer, and family history of venous thrombosis, respectively.

To summarize, our findings regarding risks of arterial and venous thrombosis are conflicting; our inability to detect an increased risk of thrombosis in individuals with MGUS is contradictory to findings reported in previous studies, where MGUS is associated with increased risks of thrombosis.[81,82] Our analysis could not detect an increased risk of a history of either arterial or venous thrombosis in individuals with MGUS at baseline, and no increased risk during follow-up was observed. We believe that the previously published investigations have been biased due to clinically detected MGUS, and it is the comorbidity in these MGUS patients that has been the true driver behind the risk of thrombosis previously observed.

The findings of an increased risk of arterial thrombosis, both at baseline and prospectively, in individuals with LC-MGUS, should be interpreted with caution. The

observed increased risks were not statistically significant in a multivariate analysis. This could be due to no real excess risk of thrombosis in LC-MGUS, and that the increased risk we detected at baseline was confounded by age or other variables insufficiently for. Another plausible explanation is that our analysis suffers from a power issue, and a larger, prospective study of more individuals and longer follow-up time is necessary before any strong conclusions can be drawn. However, the findings of an elevated risk of arterial thrombosis among individuals with LC-MGUS are especially interesting in light of the previous findings on heart disease being a prominent cause of death among individuals with LC-MGUS. The results from our analyses on thrombosis lend strength to the previously mentioned theory of an increased risk of cardiovascular disease in individuals with LC-MGUS, due to common susceptibility, shared pathogenesis, or that one condition inclines the individual towards the other.

The major limitation of our investigations detailed above was, as initially explained, the limitation of data on thrombotic events. We had access only to information on first thrombotic event, and on causes of death. Consequently, all subjects with a first incidence of any thrombosis before study baseline (and detection of MGUS or LC-MGUS), could have one or several more events of thrombosis during follow-up without our knowledge, up until date of death, when causes of death was available for all deceased individuals. This, naturally, introduced a selection bias to our study design, and we tried to compensate for this by restricting the prospective analysis only to those participants with no listed or reported prior venous or arterial thrombosis, respectively. Of course, the possibility remains of them having had an unnoted thrombosis before nine years prior to baseline, but this should then be the case for participants in all study groups (MGUS, LC-MGUS, and without MGUS) alike – unless, of course, individuals in one group such as LC-MGUS are much more prone to thrombosis, experience their events earlier than the other groups in the cohort, and are then either protected from further thrombosis due to treatment, or more prone to develop several more incidences of thrombosis. This is information we do not have access to.

A further limitation of this investigation is, as previously stated, the inability to validate individual medical records, but importantly, there are some strengths as well. The cohort design, although impaired by the data limitations discussed, is still an advantageous study design for the study of the impact of exposures on outcomes of interest. We had the possibility to adjust for several risk factors in our analyses. Furthermore, important risk factors and covariates in the model were primarily assessed through register data, and not through self-reporting, thus minimizing recall bias.

In conclusion, we found that individuals with MGUS or LC-MGUS who do not progress to a lymphoproliferative disorder still have an increased risk of death from cancer and from heart disease. The increased risk of death from heart disease is higher in LC-MGUS than in MGUS, and stems from elevated risk of death from the subgroup non-ischemic heart disease. Furthermore, we found that the risk of a history of thrombosis was higher in LC-MGUS, but not in MGUS, compared to individuals without MGUS. We found that the increased risk was due to arterial thrombosis and not venous thrombosis.

Our findings during these investigations are of clinical importance in the sense that they speak against the previously held belief that individuals with MGUS are at an increased risk of thrombosis. However, the findings that individuals with MGUS and LC-MGUS alike are at increased risk of death from both cancer and heart disease are troubling and lends further strength to the recommendations of clinical follow-up of these patients. Additionally, this is

to our knowledge the first time survival, cause of death and risk of thrombosis is studied in individuals with LC-MGUS, and the results that point towards increased risk of thrombosis in general, and arterial thrombosis in particular, should be of interest to clinical hematologists as well as the scientific community. Our findings indicate that MGUS and LC-MGUS are two clinically distinct conditions, with differing risk profiles, and that between MGUS and LC-MGUS, it is the latter condition that is the most hazardous – which further underlines the absolute need for an adequate and easily applicable definition of LC-MGUS. However, LC-MGUS is less common than MGUS, affecting only 1% of the elderly population. Bearing in mind that the risk estimates in our study are modest, the true absolute difference in survival between individuals with LC-MGUS and individuals without MGUS might not be of clinical relevance. Nevertheless, with better prediction models and a more thorough understanding of how the underlying causes of increased risk of heart disease and thrombosis interact with LC-MGUS, in the future we might be able to single out individuals with LC-MGUS who could benefit from cardiovascular risk prevention strategies.

6 FUTURE DIRECTIONS

In the future, we hope to see MGUS and LC-MGUS even further characterized, for example through a large screening study with long follow-up time. If future investigations can isolate risk factors for mortality and morbidity in these conditions, prediction models could perhaps be developed, and possibly even treatment of high risk MGUS and/or LC-MGUS could be considered.

Furthermore, we recommend scientific attention to be focused on LC-MGUS, to characterize the clinical, genetic, and biochemical profiles of this condition, with the purpose of understanding the connection to cancer, to heart disease, and to thrombosis. With a better understanding of the pathogenesis, morbidity and mortality in individuals with LC-MGUS might be decreased, or even prevented.

7 SUMMARY AND CONCLUSIONS

MGUS and LC-MGUS can be found through screening in approximately 5% and 1% in a cohort of elderly individuals (age range 59-98 years), respectively. Here we suggest a revised definition of LC-MGUS that is easily applicable, captures fewer individuals yet all with a clinically relevant condition, and can be used regardless of renal function. The proposed definition will decrease unnecessary health-care costs as well as reduce the burden of anxiety among affected patients and their families.

Individuals with MGUS and LC-MGUS appear to have inferior survival compared to individuals without MGUS, even when they do not progress to a lymphoproliferative disorder. In addition, individuals with LC-MGUS seem to have inferior survival than those with MGUS.

A preceding autoimmune disease has a negative effect on survival in MGUS and also in MM, and the effect persists regardless of M-protein isotype or M-protein concentration.

MM patients with a prior established MGUS have a better survival than those without. Among the former, a low M-protein concentration at MGUS diagnosis predicts inferior MM survival. Our findings lend support to the recommendations of a close clinical follow-up of all individuals with MGUS.

MGUS and LC-MGUS seem to be two clinically distinct conditions, with differing risk profiles. Individuals with MGUS and LC-MGUS have an increased risk of death from cancer and from heart disease. The elevated risk is greater in LC-MGUS than in MGUS, and appears to stem from non-ischemic heart disease.

Overall, individuals with MGUS do not seem to have an increased risk of venous or arterial thrombosis. However, individuals with LC-MGUS appear to have an elevated risk of arterial thrombosis. This could be indicative of an underlying genetic susceptibility to cardiovascular disease and LC-MGUS, or to the FLC overload in LC-MGUS contributing to cardiovascular morbidity.

A future more detailed characterization of the genetic, biochemical, and clinical profile of LC-MGUS, will hopefully better explain the association with cancer, heart disease, thrombosis, and inferior survival observed in this condition.

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